

Product Disposition in Periplasm Soluble Protein vs Aggregate

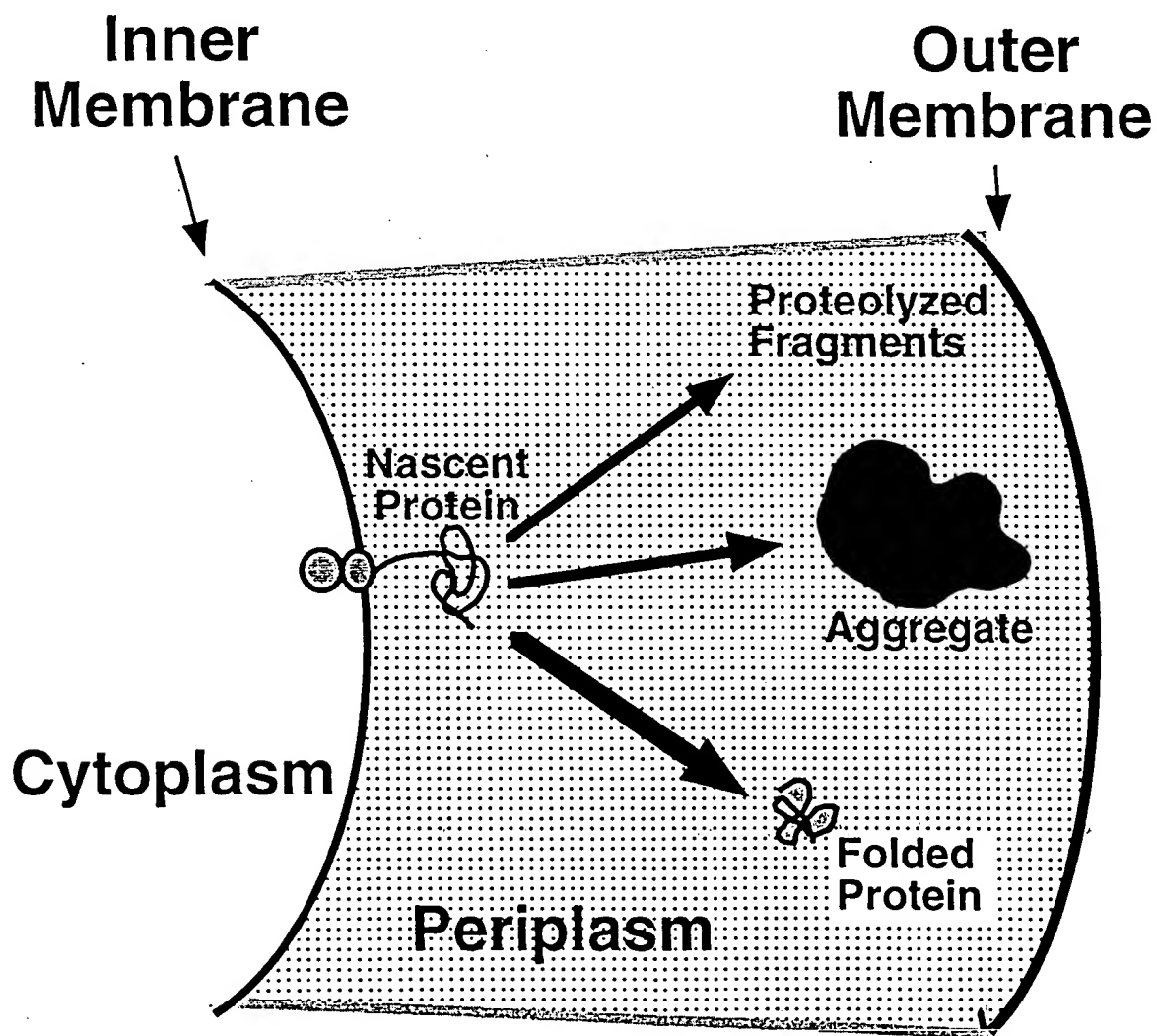


Fig. 1

Mechanical Disruption Leads to Incomplete Recovery of IGF-I Aggregates

Hart et al., Bio/Technology 12:1113 (1994)

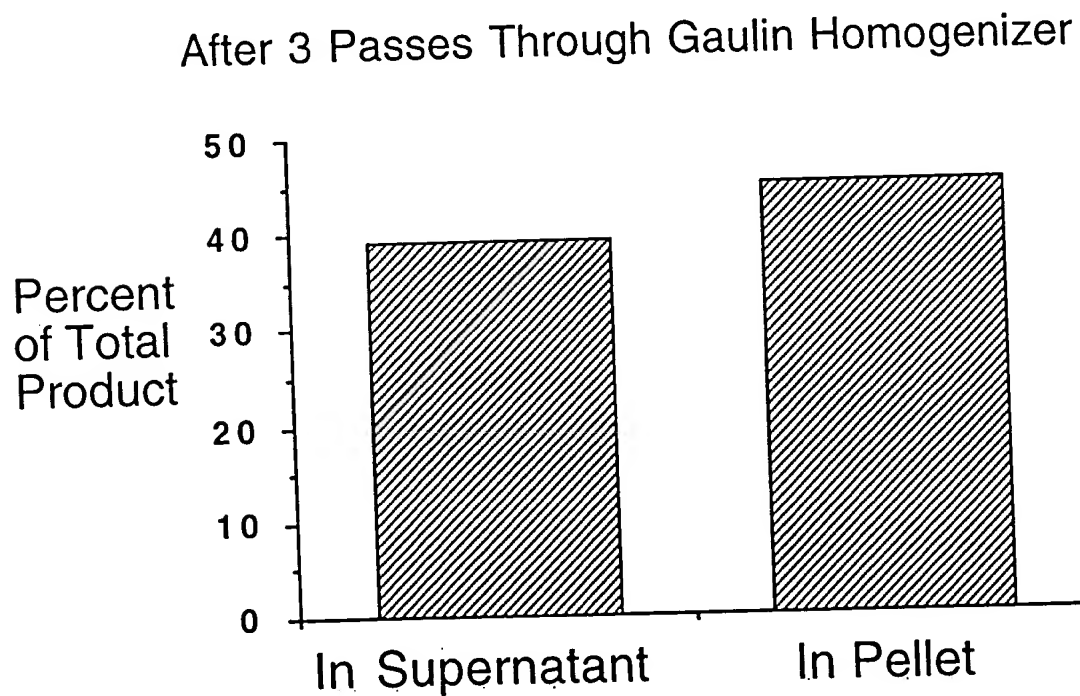


Fig. 2

pIGFLysAra Plasmid Construction

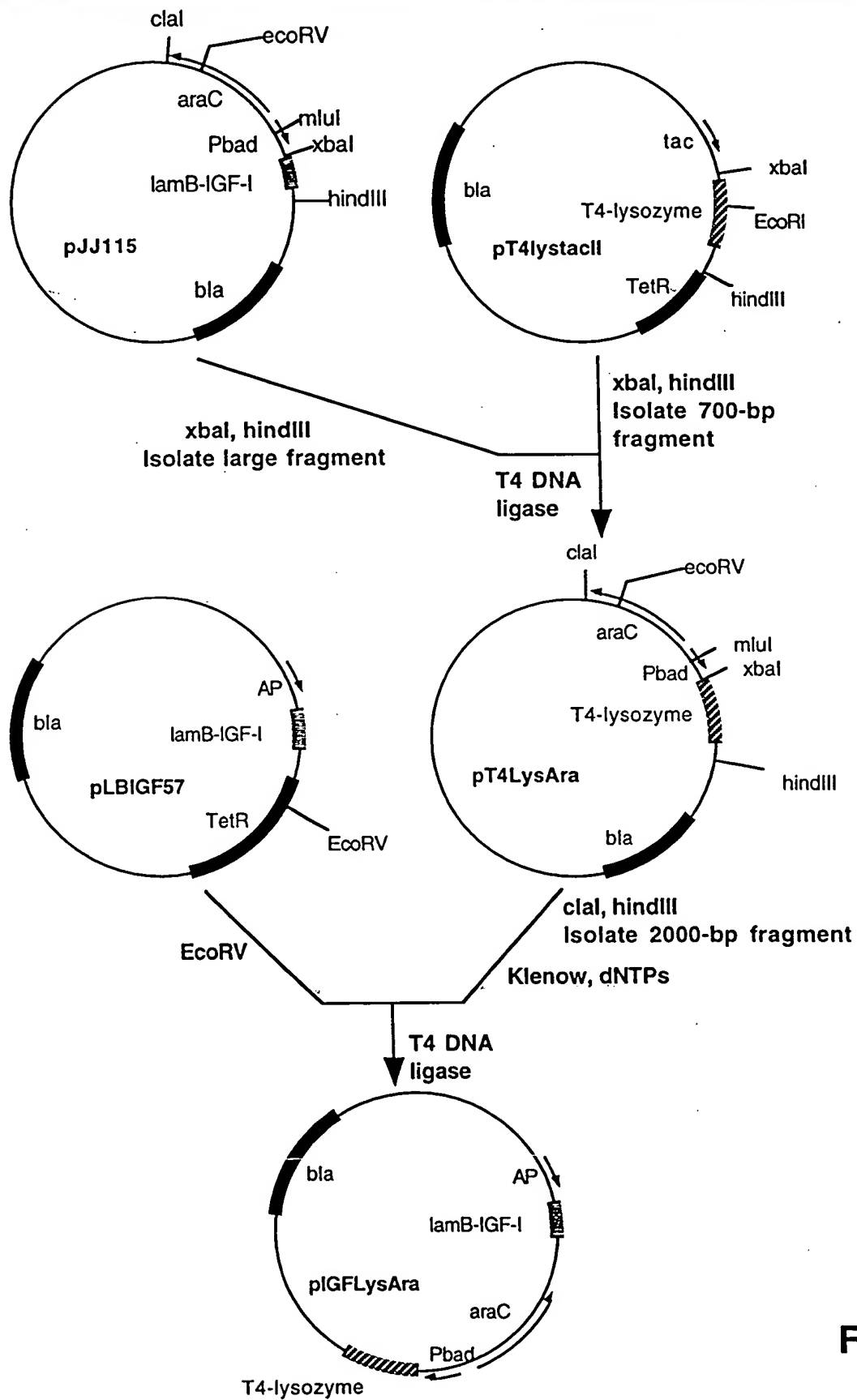


Fig. 3

Co-expression of T4-lysozyme and IGF- I by *E. coli*

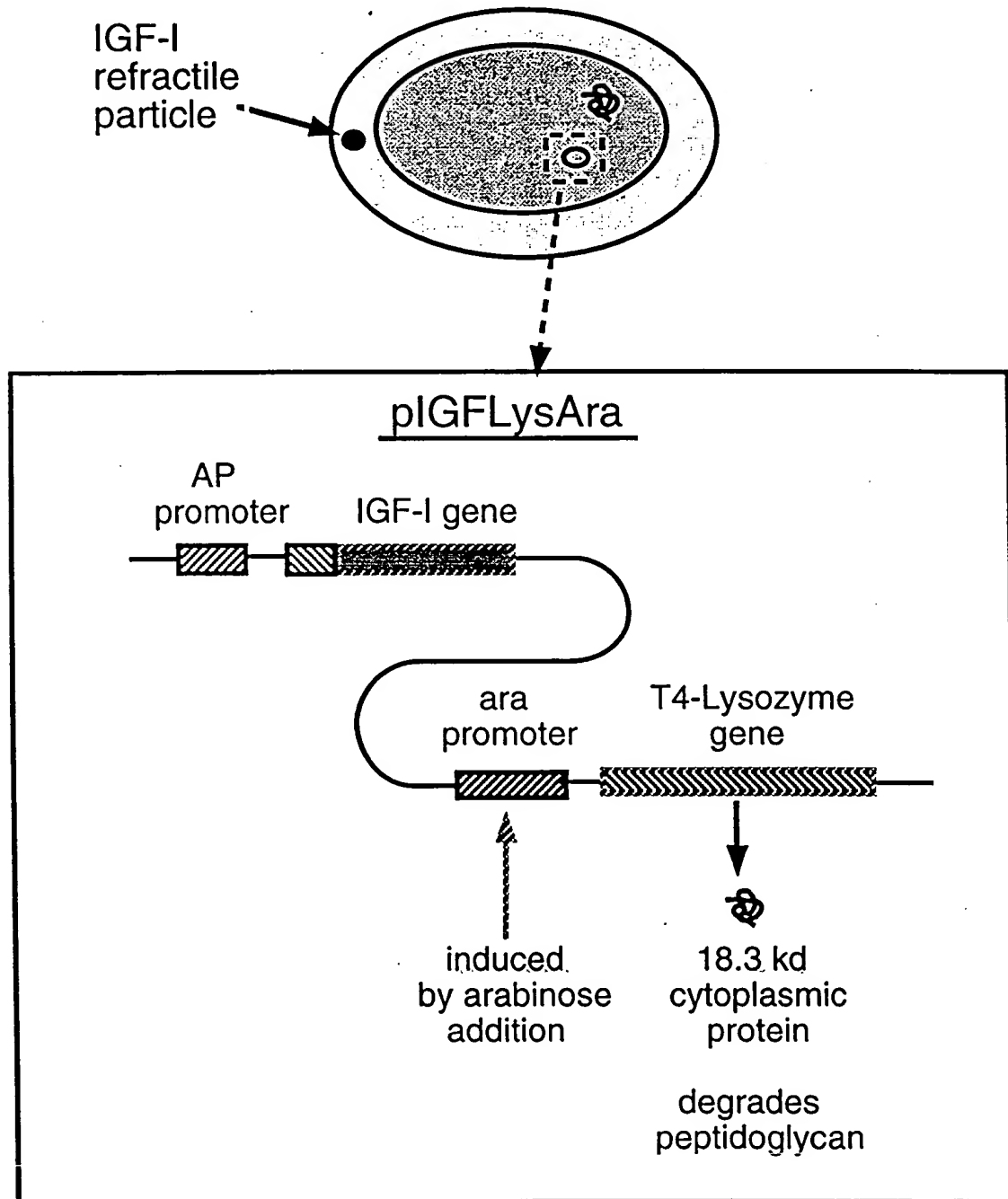


Fig. 4

rhIGF-I Fermentation Process With Co-expression of T4-Lysozyme

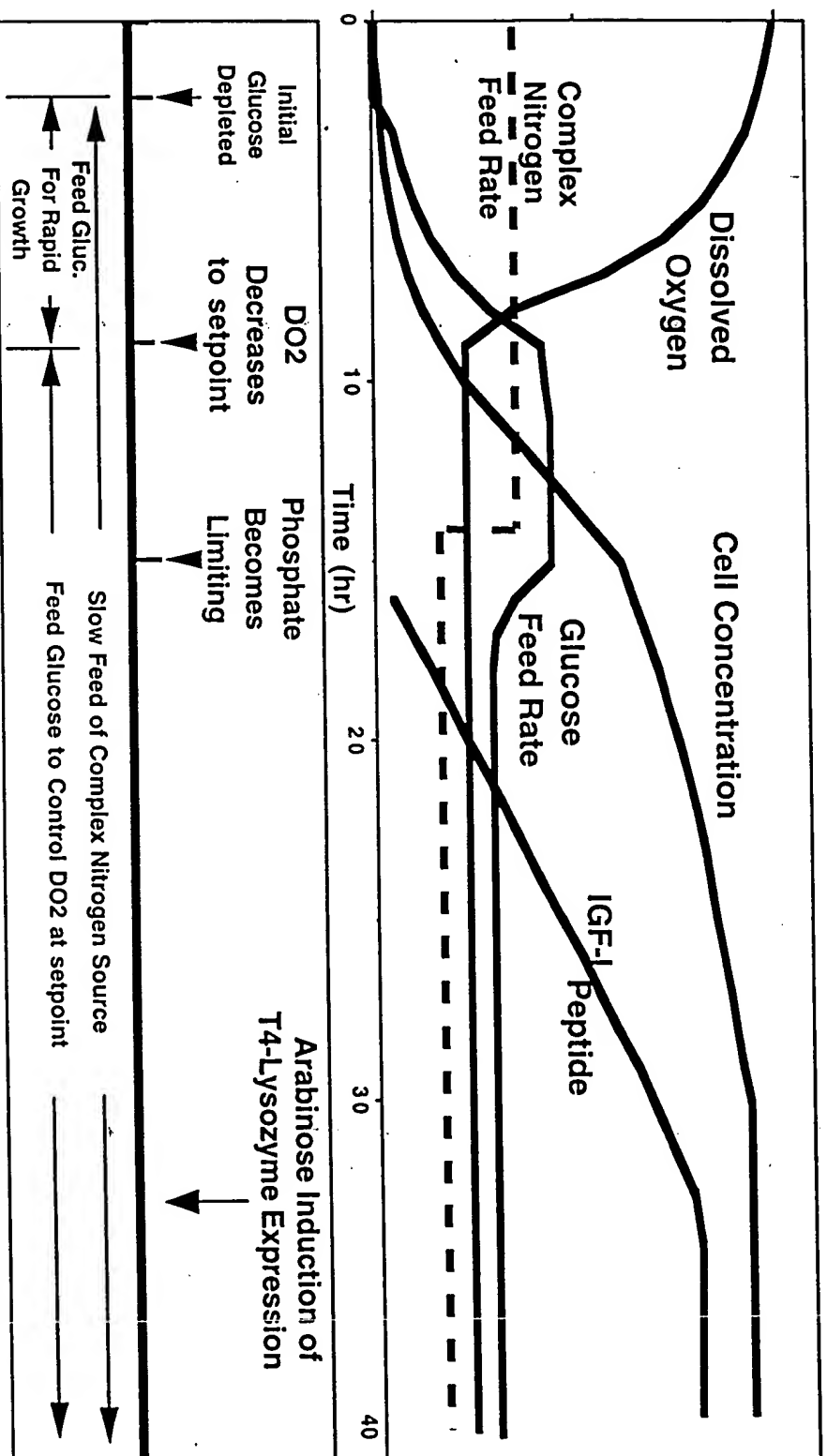
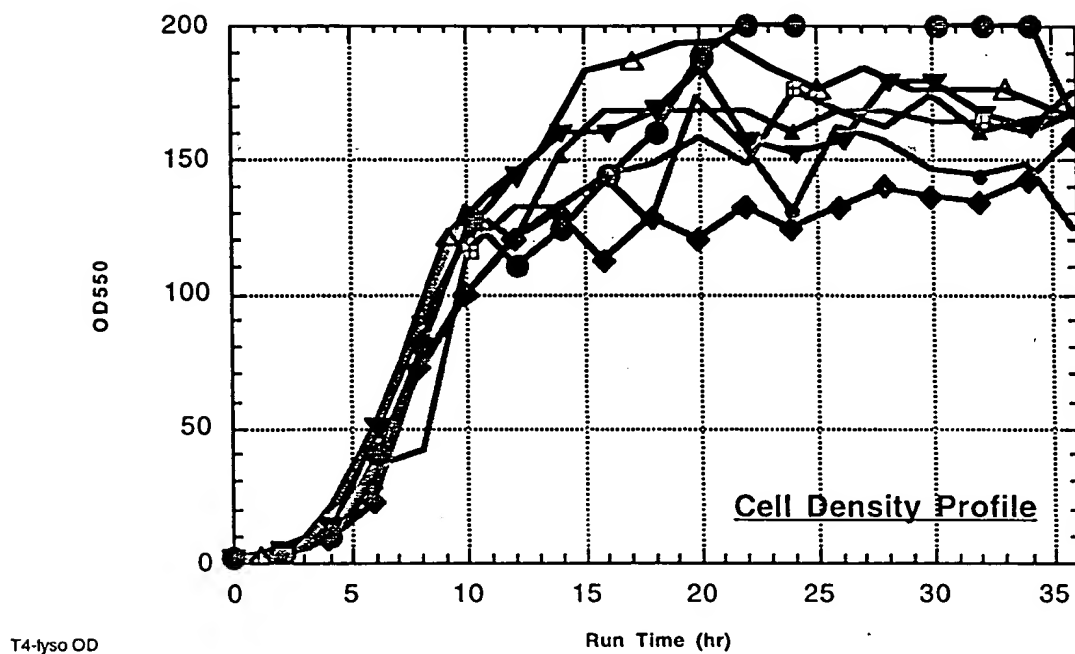


Fig. 5

Effect of Arabinose Induction for T4-lysozyme Co-expression on Cell Density Profile

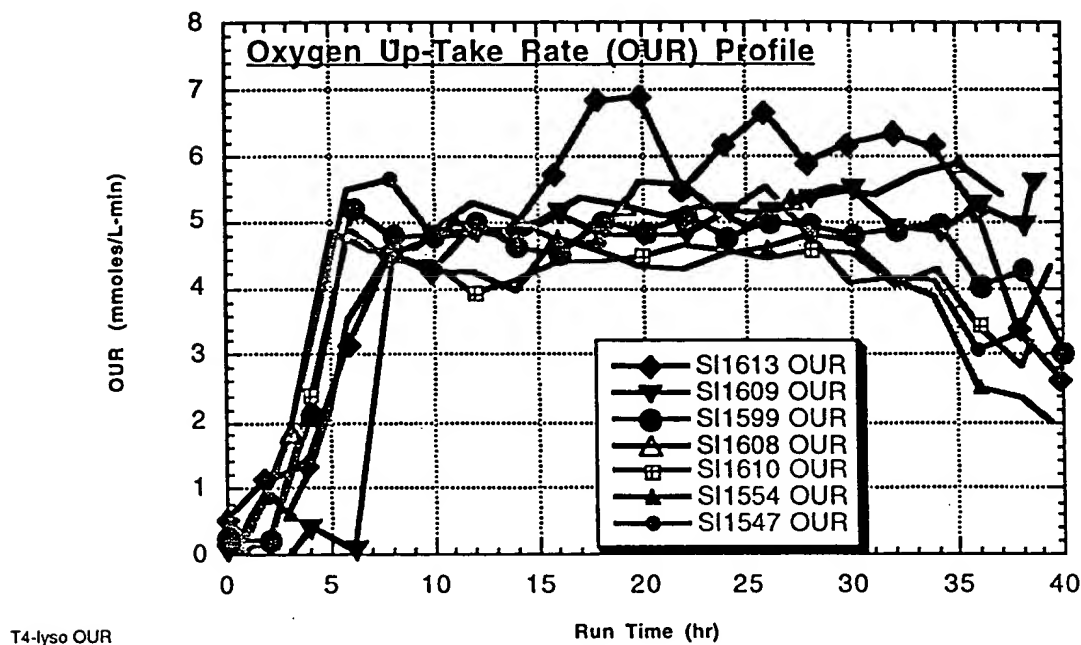


Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
SI1608	45F8/pIGFLysAra	1% arabinose induction @ 36 hrs
SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Fig. 6

Effect of Arabinose Induction for T4-lysozyme Co-expression on Cellular Respiration

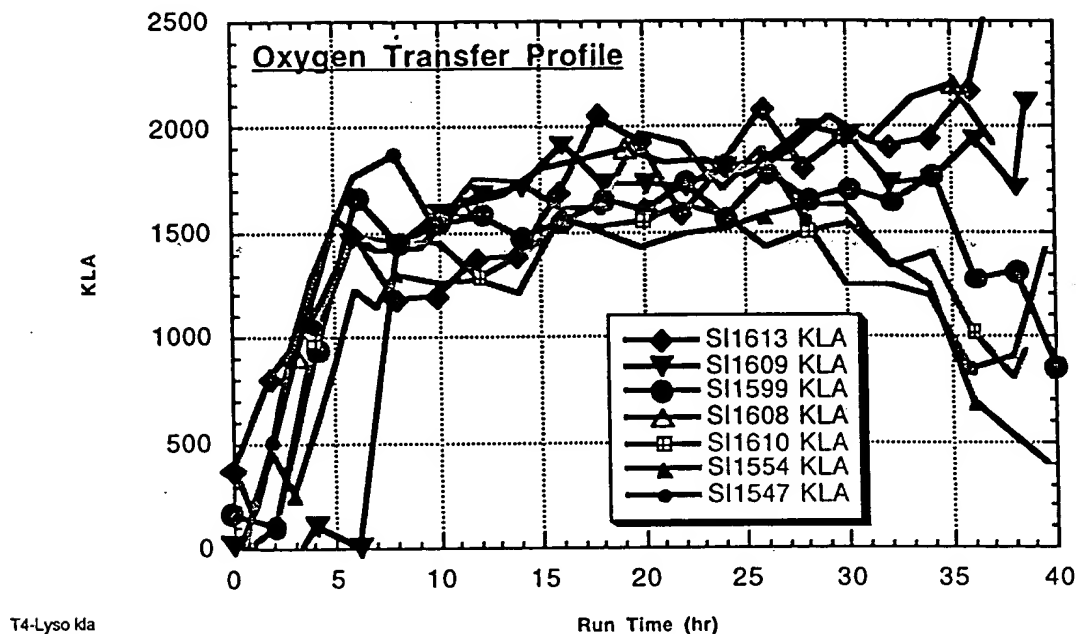


Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
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SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Fig. 7

Effect of Arabinose Induction for T4-lysozyme Co-expression on Oxygen Transfer during Fermentation



Run ID Key:

Run #	Production Organism	Test Condition
SI1613	45F8/pLBIGF57	Control organism, no arabinose induction
SI1609	45F8/pIGFLysAra	Minus arabinose induction control
SI1599	45F8/pIGFLysAra	0.1% arabinose induction @ 32 hrs
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SI1610	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1554	45F8/pIGFLysAra	1% arabinose induction @ 32 hrs
SI1547	45F8/pIGFLysAra	0.1% arabinose induction @ 24 hrs

Fig.8

Effect of T4-lysozyme Co-expression on IGF-I Accumulation Arabinose Induction of pBAD Promoter

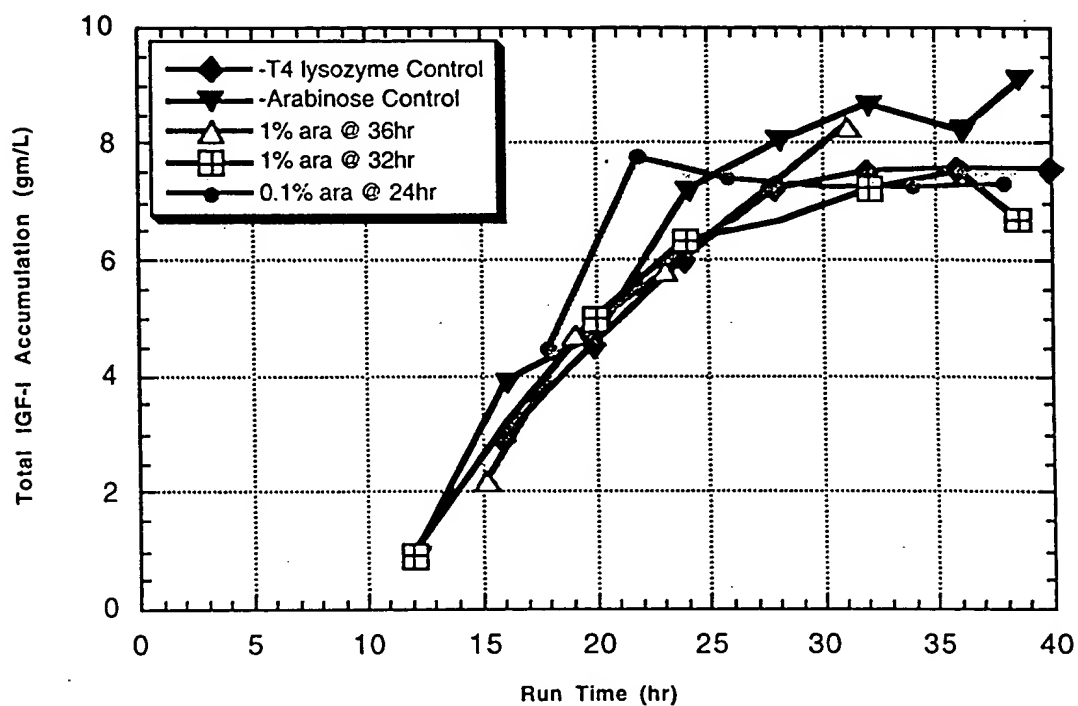


Fig. 9

Facilitating Product Isolation Procedure With T4 Lysozyme Expression

1) Induce *in vivo* T4 Lysozyme Expression

- * Sequestered in cytoplasmic compartment

2) Isolate IGF-I Aggregates

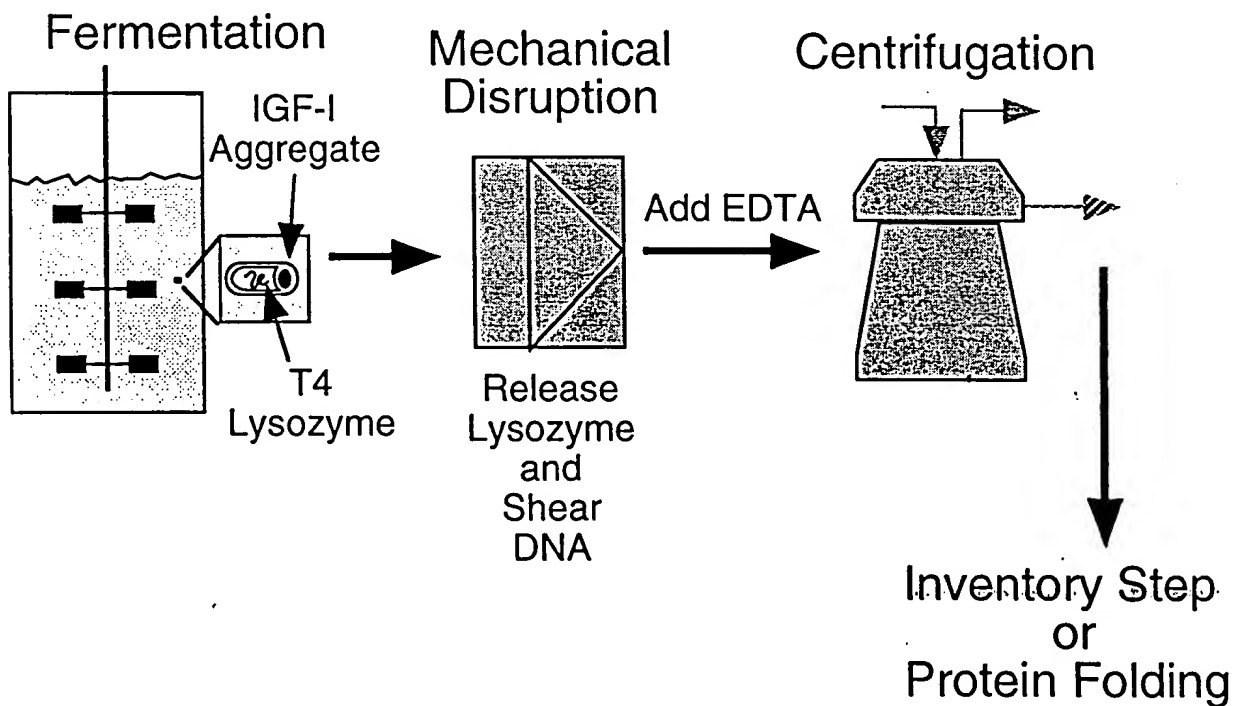
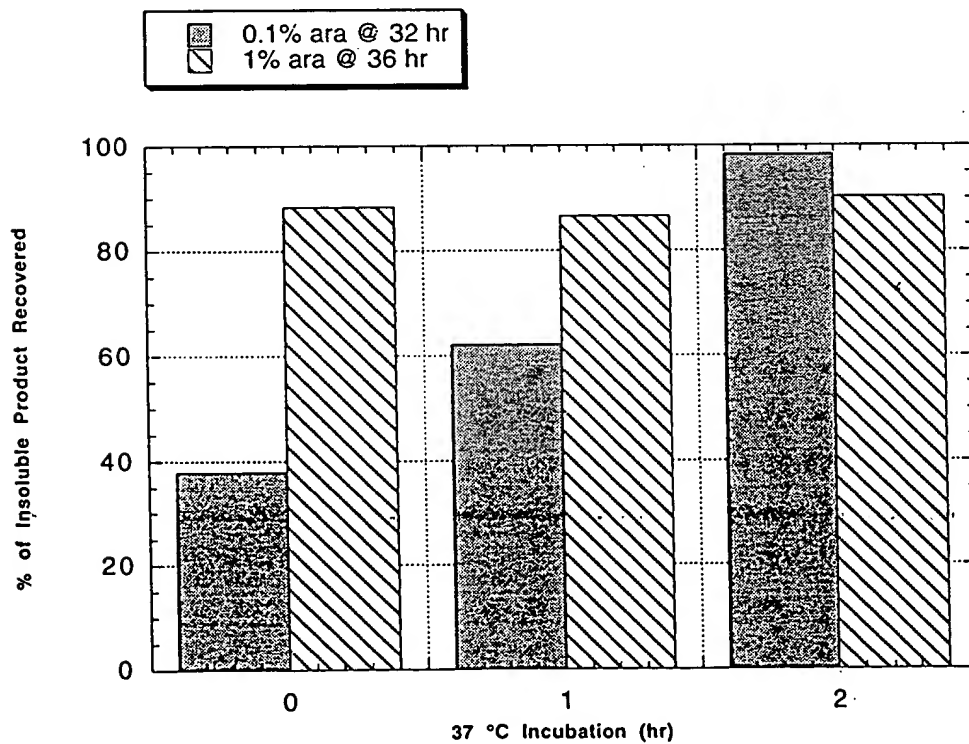
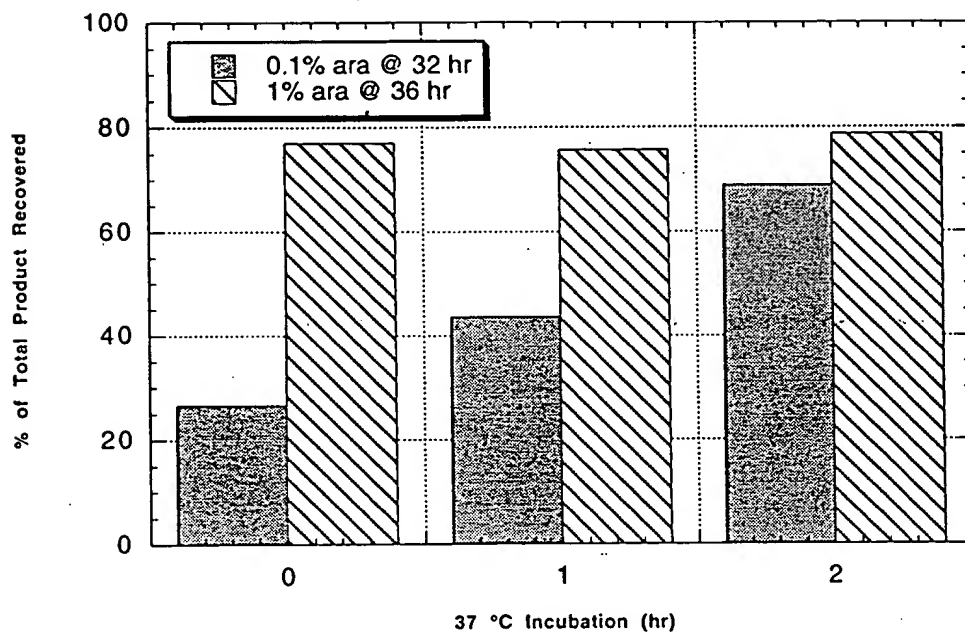


Fig. 10

FIG 11

Co-Expression of T4-Lysozyme with IGF-I for Improved RP Recovery



* RP recovered by centrifugation at 5000 rpm X 30 min in Sorval centrifugation using GSA rotor

Facilitating Product Isolation With T4 Lysozyme Co-Expression

Results:

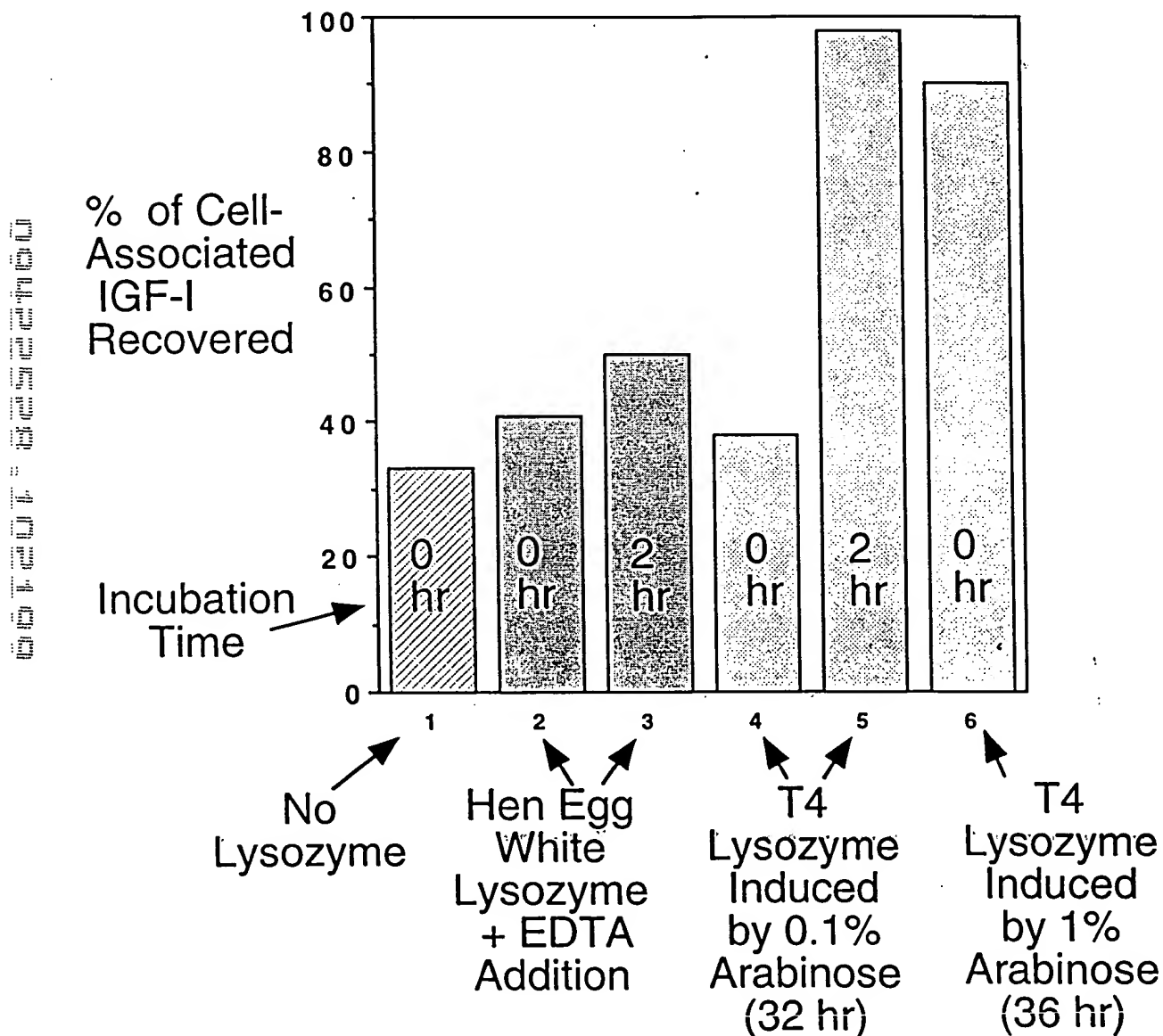


Fig. 12

Fig. 13

pJJ153 Plasmid Construction for Co-expression of ara-driven T4-Lysozyme

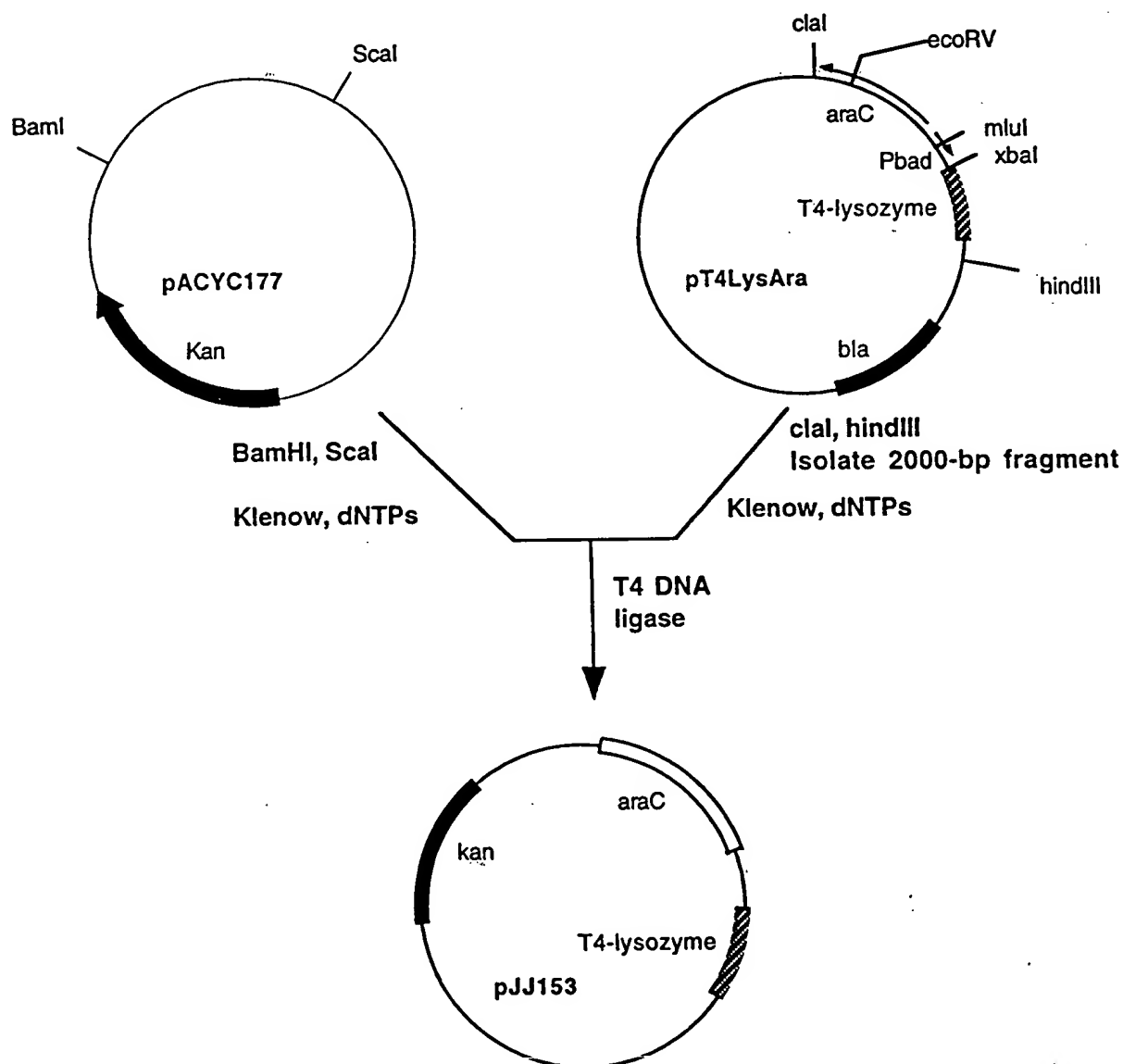
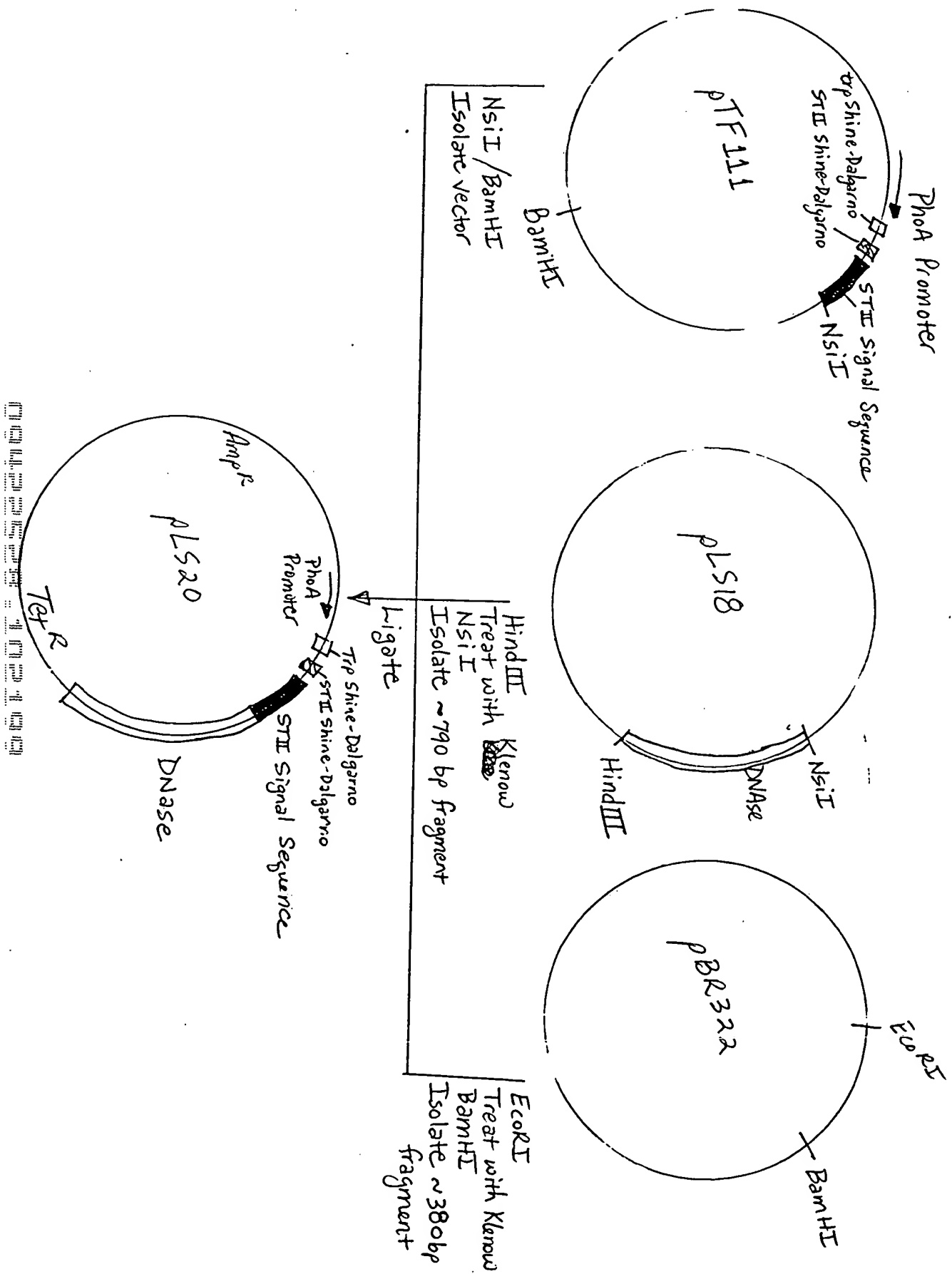


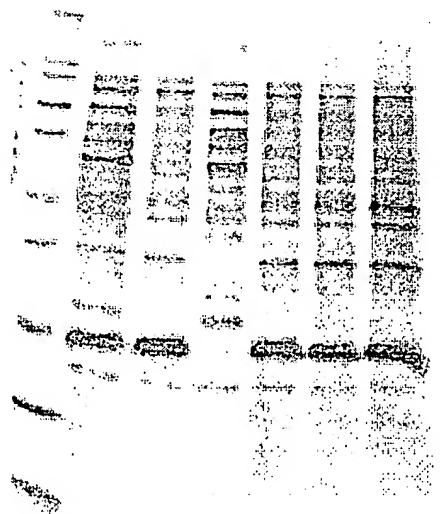
FIG. 15



RP Recovery Process Evaluation
VEGF Broth Induced for T4-Lysozyme Co-expression

Gel Analysis of RP Recovered:

MW Std
Whole Broth
Pellet
Supernatant
M3P/LE-1hr
M3P/LE-2hr
M3P/LE-20hr



← VEGF

FIG. 17

RP Recovery Process Evaluation
DNase Broth Induced for T4-Lysozyme Co-expression

Gel Analysis of RP Recovered:

